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EXAMINER
TON, THAIAN N

ART UNIT	PAPER NUMBER
1632	14

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Please find below and/or attached an Office communication concerning this application or proceeding.

Office Action Summary	Application No.	Applicant(s)	
	09/995,452	BENVENISTY ET AL.	
	Examiner	Art Unit	
	Thai-An N. Ton	1632	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If the period for reply specified above is less than thirty (30) days, a reply within the statutory minimum of thirty (30) days will be considered timely.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133).
- Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *05 June 2003* .

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *1-56* is/are pending in the application.

4a) Of the above claim(s) *18-35 and 37-47* is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *1-17,36 and 48-56* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

11)☐ The proposed drawing correction filed on _____ is: a)☐ approved b)☐ disapproved by the Examiner.

If approved, corrected drawings are required in reply to this Office action.

12)☐ The oath or declaration is objected to by the Examiner.

Priority under 35 U.S.C. §§ 119 and 120

13)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____ .

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

14)☒ Acknowledgment is made of a claim for domestic priority under 35 U.S.C. § 119(e) (to a provisional application).

a) ☐ The translation of the foreign language provisional application has been received.

15)☐ Acknowledgment is made of a claim for domestic priority under 35 U.S.C. §§ 120 and/or 121.

Attachment(s)

1) ☒ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☒ Information Disclosure Statement(s) (PTO-1449) Paper No(s) *11,18*
4) ☐ Interview Summary (PTO-413) Paper No(s). _____ .
5) ☐ Notice of Informal Patent Application (PTO-152)
6) ☐ Other: .

DETAILED ACTION

Claims 1-56 are pending. Claims 18-35 and 37-47 are withdrawn from further consideration pursuant to 37 CFR 1.142(b), as being drawn to a nonelected inventions, there being no allowable generic or linking claim. Applicant timely traversed the restriction (election) requirement in Paper No. 13.

Claims 1-17, 36 and 48-56 are under current examination.

Claim Objections

Claim 17 is objected to because of the following informalities: the claim recites "an INF receptor gene sequences". This is grammatically incorrect. Appropriate correction is required.

Claim 56 is objected to because of the following informalities: the claim does not end in a period. Appropriate correction is required.

Election/Restrictions

Applicant's election with traverse of Group I (Claims VII, claims 38, 48-56) in Paper No. 13 is acknowledged. Applicants traverse the restriction and request that the examination of the claims in groups I (1-17) and VII (36, 48-56). The traversal is on the ground(s) that the inventions embodied by groups I and VII are not distinct because substantially pure or reagent human ES cell populations cannot be made by calcium chloride transfect, as maintained by the action. Applicants argue

that although calcium chloride transfection is a known technique for transferring DNA in some cell types, the effectiveness of the technique to transfer DNA varies greatly depending upon the cell type, and that the ability of calcium chloride transfection to introduce exogenous DNA into human ES cells cannot be inferred by the technique's success in other types of cells. Applicants present the Taketo abstract as evidence that electroporation is a superior method to transfect DNA in E. coli in comparison to calcium chloride transfection. Applicants point to Figure 1 of the application to show that electroporation to be an order of magnitude less efficient for delivering DNA into human ES cells, Applicants conclude that calcium chloride transfection is, "likely to far less efficient" than the methods of the instant application. See Applicants' Response, pp. 1-2, bridging ¶.

This is not found persuasive because the requirement to show that two inventions are distinct does not require that one technique be superior or more efficient, as asserted by Applicant, than the other invention. The requirement is to show a materially different method of producing the same product. However, upon further consideration, the Examiner will examine group I (claims 1-17) with group VII (claims 36, 48-56).

Claims 18-35 and 37-47 are withdrawn from further consideration pursuant to 37 CFR 1.142(b), as being drawn to a nonelected Groups, there being no allowable generic or linking claim. Applicant timely traversed the restriction (election) requirement in Paper No. 13.

Information Disclosure Statement

Applicants' Information Disclosure Statements, filed 11/27/01 [Paper No. 8] and 6/5/03 [Paper No. 12], have been considered.

Claim Rejections - 35 USC § 112

The following is a quotation of the second paragraph of 35 U.S.C. 112:

> The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter which the applicant regards as his invention.

Claim 1, as written, is incomplete. The claim recites the introduction of a polynucleotide into a population of cells. However, for the polynucleotide to be expressed, it would be required to be operably linked to a promoter. Claims 2-10 depend from claim 1.

Claim 2, as written, is vague and indefinite. The claim recites that the expression altering sequence is *an enhancer* for modulating gene expression. It is unclear if the term enhancer is referring to an enhancer sequence, or it is a sequence that is overexpressed. Clarification and/or amendment to the claim is requested.

Claims 2-10, as written, are unclear. The claims all begin with "A method of claim # ...". This is unclear because they only refer back to one method. For example, claim 2 recites, "A method according to claim 1." There is only one method

recited in claim 1. It is suggested that the claim be re-written, "The method according to claim 1 ...".

Claim 11, as written, is unclear. The claim recites that the DNA sequence corresponds to at least one of an enhancer, a promoter, and a gene sequence to alter gene expression in the population of embryonic cell. See lines 4-5. This is unclear because a gene sequence would need to be operably linked to a promoter for expression of the gene expression. Furthermore, an enhancer or promoter would have to be specifically targeted in order to affect specific gene expression. Claims 12-17 depend from claim 11.

Claim 48, as written, is unclear. The claim recites that in the human ES cells modified by *foreign* DNA, the DNA is only expressed by selected *derivative* cells or *derivative* cells. Firstly, the term "foreign" is unclear. For example, is the DNA from another species? It is suggested the term "exogenous" be used. Secondly, the term derivative is unclear because it encompasses ES cells that remain undifferentiated and originate from the human ES cells, and cells that are differentiated from the transfected human ES cells. Clarification and/or amendment to the claim is requested. Claims 49-56 depend from claim 48.

Claim 48 recites the phrase, "foreign genetic material". The term is unclear, for example, does the DNA come from another species? It is suggested the term exogenous be used instead.

Claim Rejections - 35 USC § 102

The following is a quotation of the appropriate paragraphs of 35 U.S.C. 102 that form the basis for the rejections under this section made in this Office action:

> A person shall be entitled to a patent unless –
>
> (a) the invention was known or used by others in this country, or patented or described in a printed publication in this or a foreign country, before the invention thereof by the applicant for a patent.
>
> (e) the invention was described in (1) an application for patent, published under section 122(b), by another filed in the United States before the invention by the applicant for patent or (2) a patent granted on an application for patent by another filed in the United States before the invention by the applicant for patent, except that an international application filed under the treaty defined in section 351(a) shall have the effects for purposes of this subsection of an application filed in the United States only if the international application designated the United States and was published under Article 21(2) of such treaty in the English language.

Claims 1-4, 6, 10-13, 15, 16, 36, 48-56 are rejected under 35 U.S.C. 102(a) or 35 U.S.C. 102(e) as being anticipated by Smith *et al.* [U.S. Pat. No. 6,146,888, Reference AL of Applicants' Information Disclosure Statement, filed 3/26/03, Paper No. 11].

Claims 1-4, 6 and 10 are directed to methods of altering gene expression in a population of human ES cells comprising introducing a polynucleotide into the population of cells, wherein the polynucleotide contains a gene expression altering sequence so that gene expression in the ES cells is measurably different before and after introduction of the polynucleotide, while the cells retain their pluripotent character. In further embodiments, the expression altering sequence is an enhancer, a protein selected from a fluorescent protein (GFP, lacZ, firefly Rennila protein, luciferase, red cyan and yellow cyan protein), and an antibiotic protein (hygromycin, neomycin, zeocin, and puromycin). In further embodiments, the

polynucleotide is introduced by electroporation. Claims 11-16 are directed to methods of altering gene expression in a population of human ES cells comprising introducing a polynucleotide by electroporation or in the presence of a cationic polymer, wherein the DNA sequence corresponds to at least one of an enhancer, a promoter and a gene to alter gene expression in the population of ES cells. In further embodiments, the DNA sequence corresponds to a gene which encodes a protein selected from a fluorescent protein (GFP, lacZ, firefly Rennila protein, luciferase, red cyan and yellow cyan protein), a suicide gene (inducible apoptoic gene, herpes TK, inducible Diptheria toxin, bacterial cytosine deaminase). In further embodiments, the promoter is selected from rex-1, oct-4, oct-6, SSEA-3, SSEA-4, TRA1-60, TRA1-81, GCTM-2, alkaline phosphatase, and Hes1 promoters. The claims are further directed to cell populations comprising a substantially pure population of human ES cells containing an expression altering sequence of exogenous DNA [claims 36, 48-56].

Smith teaches the generation of genetically modified stem cells. The stem cells include both unipotential and pluripotent stem cells, embryonic stem cells, etc. See col. 2, lines 12-15. Smith teaches that the cells can contain a selectable marker which is capable of differential expression in stem cell and cells other than the desired stem cells, wherein the differential expression of the selectable marker results in preferential isolation and/or survival and/or division of the desired stem cells. They teach that the term "animal cell" embraces all animal cells, including

human cells. See col. 2, lines 1-11. In particular, Smith teaches that a positive selectable marker or a negative selectable marker may be used in transfecting the cells. For example, a foreign gene, a cellular gene, or an antibiotic resistance gene, such as neomycin. See col. 2, lines 25-29. Smith teaches that another selectable marker that may be used is a product which is toxic, such as a suicide gene, for example, herpes simplex virus TK (see col. 2, lines 46-52). They further teach that various means of introducing the selectable marker may be employed, such as transfection, viral vector, lipofection, or by electroporation. See col. 2, lines 61-64. Smith teaches that a source of cells, including stem cells, is introduced with a selectable marker construct, wherein the selectable marker is adapted to operatively link to an endogenous gene, or the introduction of a selectable marker construct, wherein the marker construct is linked to one or more gene fragments that provide differential expression. See Col. 3, lines 11-24. Smith teaches that promoter and cis-regulatory elements may be included in the expression construct. They further teach that a selectable marker may encode a cell surface antigen, or a gene product that allows for the purification of expression cells by panning of fluorescence-activated cell sorting (FACS). Smith teaches that a gene that displays a restricted stem cell expression pattern that can be used in the method, is the Oct4 gene. They teach that Oct4 transcription is highly expressed in the expanding blastocyst and in the pluripotent cells of the egg cylinder. They teach that selectable marker genes, under the control of the Oct4 promoter may be applied to

the isolation of ES cell lineages. For example, the Oct4 gene promoter can be employed to drive stem cell specific transcription of a selectable marker, such as neomycin. See col. 5.

Accordingly, Smith anticipates the claimed invention.

Claim Rejections - 35 USC § 103

The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

This application currently names joint inventors. In considering patentability of the claims under 35 U.S.C. 103(a), the examiner presumes that the subject matter of the various claims was commonly owned at the time any inventions covered therein were made absent any evidence to the contrary. Applicant is advised of the obligation under 37 CFR 1.56 to point out the inventor and invention dates of each claim that was not commonly owned at the time a later invention was made in order for the examiner to consider the applicability of 35 U.S.C. 103(c) and potential 35 U.S.C. 102(e), (f) or (g) prior art under 35 U.S.C. 103(a).

Claims 5 and 14 are rejected under 35 U.S.C. 103(a) as being unpatentable over Smith *et al.* [**U.S. Pat. No.** 6,146,888, Reference AL of Applicants' Information Disclosure Statement, filed 3/26/03, Paper No. 11] as applied to claims 1-4, 6, 8-13, 15, 16, 36 above, and further in view of Myers [**Molecular Biology and Biotechnology**, ed. Myers, VCH Publishers, Inc., 1995, pp. 165-168].

The claims are directed to methods of altering gene expression in a population of human ES cells comprising introducing a polynucleotide into the population of cells, wherein the polynucleotide contains a gene expression altering sequence so that gene expression in the ES cells is measurably different before and after introduction of the polynucleotide, while the cells retain their pluripotent character wherein the fluorescent protein is selected from green fluorescent protein, lacZ, firefly Rennila protein, luciferase, red cyan protein and yellow cyan protein.

Smith teaches the generation of genetically modified stem cells. The stem cells include both unipotential and pluripotent stem cells, embryonic stem cells, etc. See col. 2, lines 12-15. Smith teaches that the cells can contain a selectable marker which is capable of differential expression in stem cell and cells other than the desired stem cells, wherein the differential expression of the selectable marker results in preferential isolation and/or survival and/or division of the desired stem cells. They teach that the term "animal cell" embraces all animal cells, including human cells. See col. 2, lines 1-11. In particular, Smith teaches that a positive selectable marker or a negative selectable marker may be used in transfecting the

cells. For example, a foreign gene, a cellular gene, or an antibiotic resistance gene, such as neomycin. See col. 2, lines 25-29. They further teach that various means of introducing the selectable marker may be employed, such as transfection, viral vector, lipofection, or by electroporation. See col. 2, lines 61-64. They further teach that a selectable marker may encode a cell surface antigen, or a gene product that allows for the purification of expression cells by panning of fluorescence-activated cell sorting (FACS). Smith does not teach that the gene product encodes a fluorescent protein such as green fluorescent protein, lacZ, firefly Rennila protein, luciferase, red cyan protein and yellow cyan protein.

However, prior to the time the claimed invention was made, Myers teaches that bioluminescent and chemiluminescent reactions are used as analytical tools in various analytical applications, such as reporter gene studies. See p. 165, 2nd column, 1st ¶. Myers teaches that bioluminescent genes include the firefly luciferin and Renilla [see p. 165, 2nd column, lines 14-17 and #2]. Myers teaches that the gene for firefly luciferase has been cloned and is an effective reporter gene for studying transcriptional activity of cloned genomic sequences. See p. 168, #3.2.

Accordingly, in view of the combined teachings of Smith and Myers, it would have been obvious for one of skill in the art to utilize the methods of transfecting stem cells, as taught by Smith, and transfect a construct encoding a fluorescent protein, such as Rennila protein, or luciferase, with a reasonable expectation of success. One of ordinary skill in the art would have been sufficiently motivated to

make such a modification, as it was well-known in the art to use such fluorescent proteins as reporter genes and various other assays, and as supported by Myers, "Bioluminescent reactions are used as analytical tools in protein and nucleic acid blotting, in nucleic acid sequencing and hybridization assays, and in reporter gene studies ... The main advantages to these reactions are their simplicity and analytical sensitivity." See p. 165, 2nd column, 1st ¶.

Thus the claimed invention as a whole was clearly *prima facie* obvious at the time the claimed invention was made especially in the absence of sufficient, clear and convincing evidence to the contrary.

Claim 7 is rejected under 35 U.S.C. 103(a) as being unpatentable over Smith *et al.* [U.S. Pat. No. 6,146,888, Reference AL of Applicants' Information Disclosure Statement, filed 3/26/03, Paper No. 11] as applied to claims 1-4, 6, 8-13, 15, 16, 36, 48-56 above, and further in view of Fasbender *et al.* [Jour. Biol. Chem., 272(10):6479-6489, 1997].

The claims are directed to methods of altering gene expression in a population of human ES cells comprising introducing a polynucleotide into the population of cells, wherein the polynucleotide contains a gene expression altering sequence so that gene expression in the ES cells is measurably different before and after introduction of the polynucleotide, while the cells retain their pluripotent

character, wherein the polynucleotide is formulated with a cationic non-lipid polymer transfection reagent.

Smith teaches the generation of genetically modified stem cells. The stem cells include both unipotential and pluripotent stem cells, embryonic stem cells, etc. See col. 2, lines 12-15. Smith teaches that the cells can contain a selectable marker which is capable of differential expression in stem cell and cells other than the desired stem cells, wherein the differential expression of the selectable marker results in preferential isolation and/or survival and/or division of the desired stem cells. They teach that the term "animal cell" embraces all animal cells, including human cells. See col. 2, lines 1-11. In particular, Smith teaches that a positive selectable marker or a negative selectable marker may be used in transfecting the cells. For example, a foreign gene, a cellular gene, or an antibiotic resistance gene, such as neomycin. See col. 2, lines 25-29. They further teach that various means of introducing the selectable marker may be employed, such as transfection, viral vector, lipofection, or by electroporation. See col. 2, lines 61-64. Smith do not teach the formulation of the polynucleotide with a cationic non-lipid polymer transfection reagent for introduction into the stem cells.

However, prior to the time the claimed invention was made, Fasbender teach methods of transfecting various cell types utilizing complexes of cationic molecules and adenovirus, which was found to enhance gene transfer *in vitro*. See Abstract. Fasbender teach COS-1, NIH-3T3 and 9L gliosarcoma cell cultures were used for

the methods of transfection involving recombinant adenovirus vectors and various size poly-L-lysine hydrobromide polymers. The cells were subsequently infected and the uptake of the labeled adenovirus was assessed. See *Materials & Methods*. Fasbender teach that the expression of reporter genes was increased in the cultured cells when they were transfected with the combination of viral vector and cationic molecules.

Accordingly, in view of the combined teachings of Smith and Fasbender, it would have been obvious for one of ordinary skill in the art to utilize the method of stem cell transfect, as taught by Smith, by utilizing a cationic non-lipid polymer reagent, such as the poly-L-lysine hydrobromide polymers taught by Fasbender, with a reasonable expectation of success. One of ordinary skill in the art would have been sufficiently motivated to make such a modification, as it was art-recognized to optimize gene transfer techniques, as supported by Fasbender who state that, "[T]he complexes of adenovirus and cationic molecules increase the efficiency of gene transfer." See Abstract.

Thus the claimed invention as a whole was clearly *prima facie* obvious at the time the claimed invention was made especially in the absence of sufficient, clear and convincing evidence to the contrary.

Claims 8 and 9 are rejected under 35 U.S.C. 103(a) as being unpatentable over Smith *et al.* [U.S. Pat. No. 6,146,888, Reference AL of Applicants' Information

Disclosure Statement, filed 3/26/03, Paper No. 11] as applied to claims 1-4, 6, 8-13, 15, 16, 36, 48-56 above, and further in view of the Gibco BRL catalog [p. 350, 1992].

The claims are directed to methods of altering gene expression in a population of human ES cells comprising introducing a polynucleotide into the population of cells, wherein the polynucleotide contains a gene expression altering sequence so that gene expression in the ES cells is measurably different before and after introduction of the polynucleotide, while the cells retain their pluripotent character, wherein the polynucleotide is formulated with a liposomal transfection reagent (claim 8) or a cationic lipid reagent (claim 9).

Smith teaches the generation of genetically modified stem cells. The stem cells include both unipotential and pluripotent stem cells, embryonic stem cells, etc. See col. 2, lines 12-15. Smith teaches that the cells can contain a selectable marker which is capable of differential expression in stem cell and cells other than the desired stem cells, wherein the differential expression of the selectable marker results in preferential isolation and/or survival and/or division of the desired stem cells. They teach that the term "animal cell" embraces all animal cells, including human cells. See col. 2, lines 1-11. In particular, Smith teaches that a positive selectable marker or a negative selectable marker may be used in transfecting the cells. For example, a foreign gene, a cellular gene, or an antibiotic resistance gene, such as neomycin. See col. 2, lines 25-29. They further teach that various means of introducing the selectable marker may be employed, such as transfection, viral

vector, lipofection, or by electroporation. See col. 2, lines 61-64. Smith do not teach specifically the formulation of the polynucleotide with a liposomal transfection reagent (claim 8) or a cationic lipid reagent (claim 9) for the introduction of the polynucleotide into a population of cells.

However, prior to the time the claimed invention was made, the Gibco BRL catalog teaches LIPOFECTIN®, which is a liposomal formulation of a cationic lipid which is used to transfect a wide variety of cells, including human cells. See 1st ¶.

Accordingly, in view of the combined teachings of Smith and the Gibco BRL catalog, it would have been obvious for one of skill in the art to utilize the methods of transfecting human ES cells, as taught by Smith, by using a transfection reagent, such as LIPOFECTIN®, with a reasonable expectation of success. One of ordinary skill in the art would have been motivated to make such a modification, as it was an art-recognized goal to optimize transfection techniques of mammalian cells, and, as supported by the Gibco BRL catalog, that the LIPOFECTIN® reagent is a more efficient method of transfecting cells than calcium phosphate or DEAE-dextran transfection methods.

Thus the claimed invention as a whole was clearly *prima facie* obvious at the time the claimed invention was made especially in the absence of sufficient, clear and convincing evidence to the contrary.

Claim 17 is rejected under 35 U.S.C. 103(a) as being unpatentable over Smith *et al.* [U.S. Pat. No. 6,146,888, Reference AL of Applicants' Information Disclosure Statement, filed 3/26/03, Paper No. 11] as applied to claims 1-4, 6, 8-13, 15, 16, 36, 48-56 above, and further in view of Pascolo *et al.* [**J. Exp. Med.**, 185:2043-2051, 1997].

The claim is directed to methods of altering gene expression in a population of human ES cells comprising introducing a polynucleotide into the population of cells by electroporation or in the presence of a cationic polymer, a DNA sequence corresponding to at least one of an enhancer, a promoter and a gene, in an amount to permit cells containing the DNA sequence to be distinguished from cells absent the DNA sequence, wherein the DNA sequence causes a knockout of a genomic sequence selected from beta-2 microglobulin, HLA-1, HLA-2, or an INF receptor gene sequence.

Smith teaches the generation of genetically modified stem cells. The stem cells include both unipotential and pluripotent stem cells, embryonic stem cells, etc. See col. 2, lines 12-15. Smith teaches that the cells can contain a selectable marker which is capable of differential expression in stem cell and cells other than the desired stem cells, wherein the differential expression of the selectable marker results in preferential isolation and/or survival and/or division of the desired stem cells. They teach that the term "animal cell" embraces all animal cells, including human cells. See col. 2, lines 1-11. In particular, Smith teaches that a positive

selectable marker or a negative selectable marker may be used in transfecting the cells. For example, a foreign gene, a cellular gene, or an antibiotic resistance gene, such as neomycin. See col. 2, lines 25-29. They further teach that various means of introducing the selectable marker may be employed, such as transfection, viral vector, lipofection, or by electroporation. See col. 2, lines 61-64. Smith do not teach the knocking out of a genomic sequence in the ES cells, wherein the genomic sequence is selected from beta-2 microglobulin, HLA-1, HLA-2, or an INF receptor gene sequence.

However, prior to the time the claimed invention was made, Pascolo teach the generation of mice which are double knockouts of H-2D^b and mouse beta2 microglobulin and express human beta2 microglobulin and HLA-A2.1 monochains. See *Abstract* and p. 2043, col. 1-2, bridging ¶. In particular, Pascolo teach the various plasmids that were used to knock out the endogenous genes [see *Materials & Methods*] and the electroporation of the plasmids into mouse ES cells. See p. 2044, 1st column, *Cells & Transfectants*.

Accordingly, in view of the combined teachings, it would have been obvious for one of ordinary skill in the art to utilize the methods of transfecting human ES cells, as taught by Smith, to knockout a genomic sequence, such as beta-2 microglobulin, as taught by Pascolo, with a reasonable expectation of success. One of ordinary skill would have been sufficiently motivated to make such a modification, as it was an art-recognized technique to knock-out endogenous genes

to analyze gene expression and, and that in generating the double knockout H-2D^b /mouse beta2 microglobulin, Pascolo states, "This should facilitate the study of HLA class I-restricted responses compared to classical transgenic mice. One might home that the information gained with these animals will be of human relevance." See p. 2050, 2nd column, lines 4-7.

Thus the claimed invention as a whole was clearly *prima facie* obvious at the time the claimed invention was made especially in the absence of sufficient, clear and convincing evidence to the contrary.

Claims 1-4, 6, 10-12, 15, 16, 36, 48, 52, 54 and 56 are rejected under 35 U.S.C. 103(a) as being unpatentable over Thomson [**Science**, 282:1145-1147 (1998)] when taken with Bradley *et al.* [**U.S. Pat. No.** 5,614,396, published March 25, 1997].

The claims are directed to methods of altering gene expression in a population of human ES cells comprising introducing a polynucleotide into the population of cells, wherein the polynucleotide contains a gene expression altering sequence so that gene expression in the ES cells is measurably different before and after introduction of the polynucleotide, while the cells retain their pluripotent character and

Thomson teach human blastocyst-derived pluripotent cell lines that can proliferate in an undifferentiated state for 4-5 months. See Abstract. In particular, Thomson teach that human embryos were cultured to the blastocyst stage and inner

cell masses were isolated and cultured. The resulting cells had morphology similar to that of rhesus monkey ES cells, expressed high levels of telomerase activity and expressed cell surface markers that characterize undifferentiated nonhuman primate ES and human EC cells. See p. 1145, col. 2-3. It was found that the cells produced teratomas after injection into SCID mice, and that the teratomas included cells of the endoderm, mesoderm and ectoderm. See p. 1146, 1st column, 2nd full ¶. Thomson do not teach the transfection of the human pluripotent stem cells with a polynucleotide.

However, prior to the time the claimed invention was made, Bradley teach methods of transfecting pluripotent human embryonic stem cells. See col. 8, lines 3-24 and lines 34-44. In particular, Bradley teach detectable markers that may be introduced in the stem cells include a suicide gene, for example, a tk gene (particularly that of herpes simplex), or genes which confer resistance to antibiotics (such as hygromycin). See col. 15, lines 11-36. Bradley particularly teach that for the ES cells can be transfected by electroporation. See Example 1.

Accordingly, in view of the combined teachings of Thomson and Bradley, it would have obvious for one of ordinary skill in the art to transfect the human pluripotent stem cells, as taught by Thomson, by the method taught by Bradley, with a reasonable expectation of success. One of ordinary skill in the art would have been sufficiently motivated to make such a modification, as supported by

Bradley, that transgenic pluripotent stem cells can be easily selected, for example, if they express a selectable marker. See col. 4, lines 26-38.

Thus the claimed invention as a whole was clearly *prima facie* obvious at the time the claimed invention was made especially in the absence of sufficient, clear and convincing evidence to the contrary.

Conclusion

No claim is allowed.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Thái-An N. Ton whose telephone number is (703) 305-1019. The examiner can normally be reached on Monday through Friday from 8:00 to 5:00 (Eastern Standard Time), with alternating Fridays off. Should the examiner be unavailable, inquiries should be directed to Deborah Reynolds, Supervisory Primary Examiner of Art Unit 1632, at (703) 305-4051. Any administrative or procedural questions should be directed to William Phillips, Patent Analyst, at (703) 305-3482. Papers related to this application may be submitted to Group 1600 by facsimile transmission. Papers should be faxed to Group 1600 via the PTO Fax Center located in Crystal Mall 1. The faxing of such papers must conform with the notice published in the Official Gazette, 1096 OG 30 (November 15, 1989). The CM1 Fax Center number is (703) 872-9306.

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